United States
Department of
Agriculture

Forest Service

Forest Pest
Management

Davis, CA

STUDY PLAN

OFF-SITE MOVEMENT OF *Bacillus thuringiensis* SPRAY APPLIED IN COMPLEX FORESTED TERRAIN

APRIL 30, 1991

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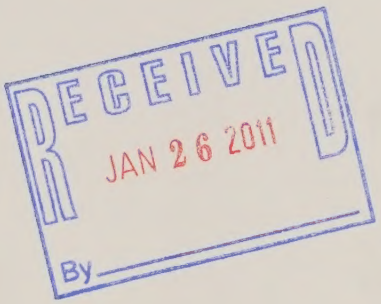

Cooperators:

USDA Forest Service
U.S. Army Dugway Proving Ground
Utah Department of Agriculture
University of California (Davis)
Continuum Dynamics, Inc.
Entotech, Inc. (NOVO)

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PREFACE

This study plan covers field procedures to be followed in conduct of an off-site spray movement study during May-June 1991. The study will be conducted in Parleys Canyon, Salt Lake County, near the Mountain Dell Golf Course in conjunction with the 1991 Utah gypsy moth eradication project. A biological pesticide Bacillus thuringiensis will be applied by helicopter. The study is in follow-up to recommendations from Program WIND, a U.S. Department of Agriculture-Forest Service (USDA-FS) and U.S. Army cooperative meteorological and computer model study; and recommendations from the USDA-FS national steering committees to further evaluate and technology transfer of computer models that predict the movement and deposition of sprays released from aircraft. Scientists from the USDA-FS and U.S. Army, in cooperation with Utah State Department of Agriculture; University of California (Davis Campus); Continuum Dynamics, Inc.; and Entotech, Inc. The cooperators will participate in one or more aspects of this study. A concurrent study will be conducted by U.S. Army scientists in Parleys Canyon to quantitate spray penetration and deposition on Gambel oak trees. The aerially applied spray, to be sampled during this study, will be applied by helicopter under operational conditions of the eradication project. No special spray treatment will be applied and no tracers will be added to the spray tank mix for the benefit of this study or the spray penetration study. This study plan may be modified as needed and as agreed to by the cooperators.

INTRODUCTION

Off-target movement of pesticides from forest spray operations has been a concern since aircraft were first used to spray trees (Neillie and Houser 1922). The concern primarily centers on potential environmental impact of pesticides on non-target species. Biological pesticides, such as Bacillus thuringiensis (B.t.), are not exempt from this concern. Assessing potential environment impact of pesticides first requires quantitative data on the amount of pesticide that moves and deposits off the target site, followed by conducting environmental impact evaluations. Off-target movement also represents an inefficient use and economic waste of pesticides. For these reasons data are also needed to quantitate off-target movement that may lead to improving efficiency and efficacy of aerial spray operations. Predictions of the Forest Service Cramer-Barry-Grim (FSCBG) aerial spray model (Bjorklund et al. 1989), a computer model that predicts travel and deposition of aerial sprays, has been compared favorable to several sets of observed field data and reported by GCA Corporation (1971); Boyle et al. (1975); Dumbauld et al. (1976); Dumbauld et al. (1977); Rafferty et al. (1987), Rafferty et al. (1988), Rafferty et al. (1989), and Teske et al., in press (1991).

Sampling off-target drift of pesticides in forests and over complex topography presents technical challenges. Researchers have had relatively few opportunities to obtain such data in forested, complex terrain. In those situations where they have tried, results have been somewhat disappointing due to a variety of reasons including type of samplers and tracers used, sample contamination, and inadequate weather monitoring. Spray drift resulting from treatment of coniferous seed orchards has been reported by Barry et al. (1983); however the reported tests were conducted in relatively flat terrain.

OBJECTIVE

The objective is subdivided into three tasks as listed below:

Task 1 - to evaluate the Wagner, Rotorod, High Volume, Kromekote card, and Mylar sheet samplers, sampling equipment, and field methods for detecting and measuring air concentration, impaction, and deposition resulting from off-site movement of B.t. pesticide sprays in complex terrain.

Task 2 - to quantitate, subject to success of Task 1, off-site movement of FORAY 48B as measured from air drawn, impaction, and deposition samplers.

Task 3 - to compare FSCBG predictions of air concentration and deposition to observed data obtained from field samplers; and conduct sensitivity analyses of the input variables measured inputs to the FSCBG.

SCOPE

The off-site movement study and treatment site is located in Salt Lake County, Utah, R2E, T1S, Sections 2,3,4,9,10, and 11. These sections, composed of public and private lands, are located in Parleys Canyon, along Interstate 80. The terrain is mountainous and partially covered with Gambel oak. The site is ideally suited for the study due to topography, channeling of drainage winds, and physical access.

The treatment site, designated as the Alexander Creek Spray Block (SL-3), consists of 2,080 acres (Figure 1). It will be treated by a helicopter (Hughes 500D and/or Bell 206B-111) applying FORAY 48B in May-June 1991 to eradicate gypsy moth, a defoliator of oak and other deciduous trees. After the first spray is applied, treatment will be repeated two additional times at five day intervals thus providing an opportunity for three replicated trials. Off-site movement studies will be conducted only in the vicinity of Block SL-3. Spray moving down slope of the treatment area will be sampled by a variety of samplers positioned downwind to approximately 2 miles. Decisions on specific location of samplers will be made after release of smoke. Weather will be monitored by three EMCOT weather stations. Several organizations will cooperate in this study. A dry run will be conducted on or about May 15, 1991 to practice field procedures and to coordinate timing.

METHODS

Application

The SL-3 block of 2,080 acres will be treated operationally with the B.t. pesticide FORAY 48B undiluted applied by helicopter at 0.5 gallons per acre. A total of 1,040 gallons will be applied. Success of the study is dependent upon an organized drainage wind that results from nighttime cooling of slopes. To increase potential for a successful study the Treatment Supervisor's will be requested to:

1. Begin application of SL-3 at first light when the pilots believe it is safe to fly and complete spraying before upslope winds begin.

2. Avoid spraying SL-3 if cloud cover precludes surface cooling. Drainage wind depends upon surface cooling and that might not happen if clouds hold warm air near the surface.

3. Complete spraying in SL-3 in one day.

4. Spray the adjacent block Mountain Dell (SL-4) on different days to avoid contamination of samplers that are sampling spray movement from SL-3.

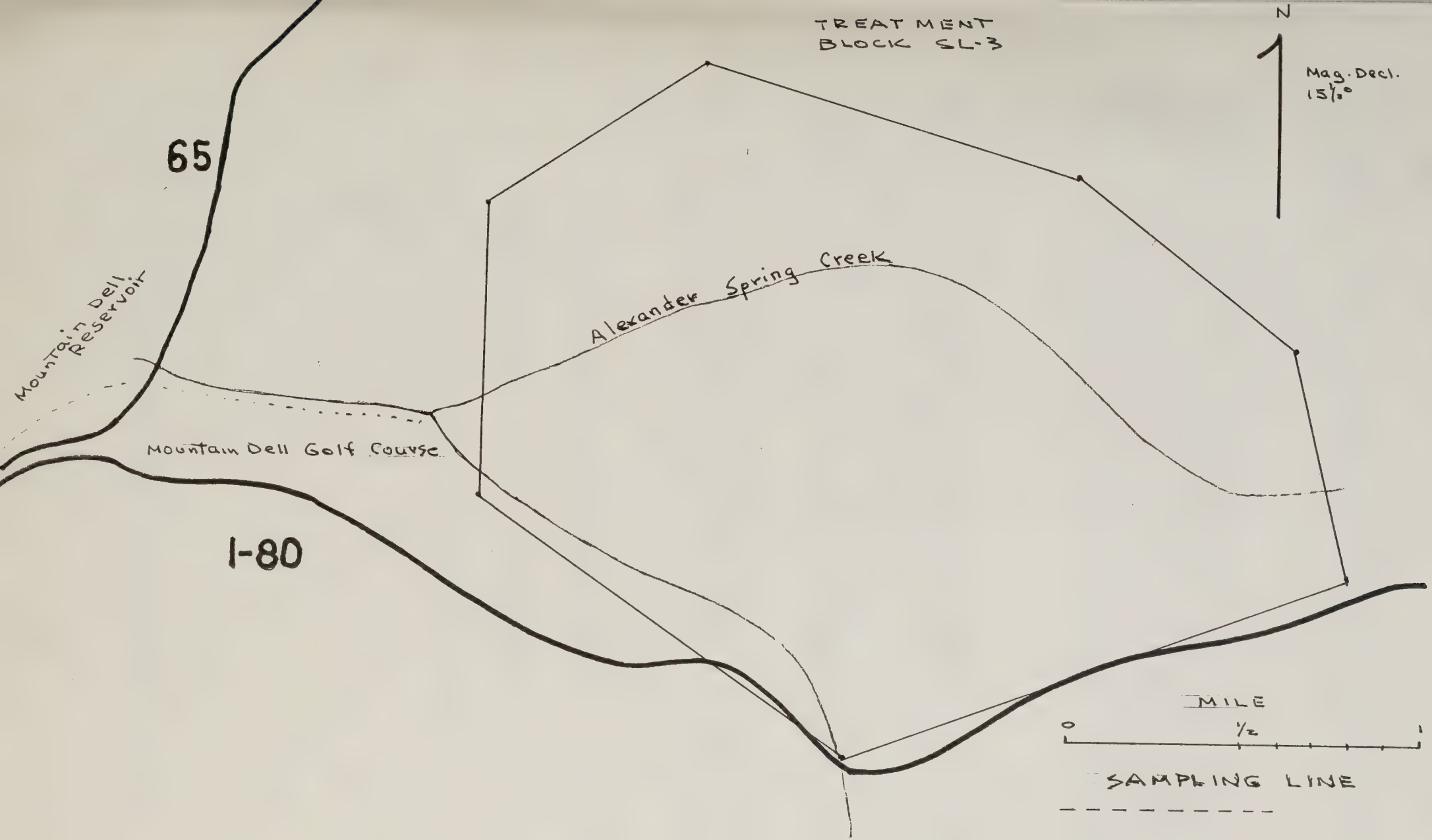

Figure 1. Alexander Creek treatment block.

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JWB

Field data collection requirements are listed in the paragraph Field Data Requirements. It is requested that the information listed be provided each day that Block SL-3 is treated.

Spray Material (Tank Mix)

FORAY 48B (see attached pesticide label in Appendix) is a commercial formulation of Bacillus thuringiensis Berliner var. Kurstaki, with a potency of 12,600 infectious units per milligram equivalent to 48 billion international units (BIU) per gallon. It will be applied undiluted at the rate of 0.5 gallons (24 BIU) per acre. No tracers or other additives will be added to the tank mix. The material has a relatively low rate of volatility. Wind tunnel testing of the atomization under conditions (atomizer, air speed, and application rate) approximating the anticipated operational conditions, is provided in Table 1.

Sampling

Off-site movement of FORAY 48B will be sampled downwind to approximately 2.0 miles for air concentration with the Wagner sampler (Figure 2) aspirating and "U"-shaped brass Rotorod samplers (Figures 3-4-5), rotating; for impaction with the "U"-shaped brass Rotorod[1] static (not rotating); and for deposition with white Kromekote cards and Mylar sheets. Other types of samples may be used if necessary to meet study objective. Twenty sampling stations (10 pairs) will be positioned at 10 locations along a line that runs downwind of the treatment block approximately 2 miles. Approximate location of the sampling stations is shown in Figures 1, 6, and 7.

Samplers will be positioned and set-out the morning of treatment, and picked-up after the spray operations and spray cloud passage is complete. The Test Officer in consultation with the Project Meteorologist will decide when to activate and deactivate the samplers.

Each sampling station (Figure 8) will consist of the following:

1. 6 each Wagner samplers, operated sequentially (one at a time) at 12.5 liters of air per minute (lpm).

2. 2 each "U"-shaped Rotorods rotated clockwise at 2400 rpm, potentially sampling 120 (lpm).

3. 2 each "U"-shaped Rotorods positioned statically (not rotating).

4. 2 each Kromekote cards.

5. 2 each Mylar sheets.

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[1]*Rotorod[R] is a registered trademark of Metronics Associates, Inc.

[illegible] requirements are listed in the procedure [illegible] Fluid Logic
[illegible] expected that the information listed [illegible] apply

[illegible]

[illegible] extended [illegible] in Appendix [illegible] of Bacillus thuringiensis, Berliner var. [illegible], with a potency [illegible] infectious units per milliliter equivalent to [illegible] international [illegible] per gallon. It will be applied undiluted at the rate [illegible] BIU) per acre. [illegible] tracers [illegible] with [illegible] The water [illegible] relatively [illegible] the [illegible] wind [illegible] of the simulated [illegible] air speed, and [illegible] approximating the [illegible] operational conditions, [illegible] in [illegible] 1.

[illegible]

[illegible] movement of [illegible] will be sampled downwind [illegible] approximately [illegible] for air concentration with [illegible] Wagner samplers (Figure [illegible]) rotating and [illegible] Rotorod samplers (Figures [illegible]), [illegible] Rotorod stationary [illegible] cards and [illegible] sheets. Other [illegible] samplers [illegible] to meet study objectives [illegible] sampling stations [illegible] locations [illegible] miles, approximate location [illegible] in Figures 1, 6, and 7

[illegible] will be positioned and set-out the morning of [illegible] the spray operations and [illegible] in consultation with the Project [illegible] and deactivate the samplers

[illegible] sampling station (Figure 8) will consist of the following

[illegible] 6 each Wagner samplers, operated [illegible] liters of air per minute (lpm)

2 each "U"-shaped Rotorods rotated clockwise [illegible] sampling [illegible] (lpm)

2 each "U"-shaped Rotorods positioned stationary [illegible]

2 each Kromekote cards

2 each Mylar sheets.

The Wagners and Rotorods will be elevated at 1.5 meters above the ground, and the Kromekote cards and Mylar sheets will be placed in plastic holders and in-turn these will be placed on boards at ground level. The board will insulate the samplers from moisture and help to reduce shielding from plants. The samplers will be activated prior to anticipated spray cloud arrival. Further details will be coordinated with the U.S. Army Dugway Proving Ground (DPG) designated Test Officer.

In addition to the above described sampling, a High Volume (HV) sampler will be operated at sampling station pairs 9-10 and station 19-20. The HV will sample at 1,000 lpm and contain a phosphate buffered collecting fluid. The HV will be powered by a 110-volt AC gasoline powered generator. The HV intake will also be positioned at 1.5 meters above ground.

Table 1. Atomization of FORAY 48B from Beecomist as measured in the University of California (Davis) wind tunnel.

Nozzle	BEECOMIST	Slice Rate	1 MHz
Angle to Airstream	0 degrees	AVG	100
Spray Pressure	15 psi	DFM	1 cm.
Airspeed	60 mph	BAR	1.5
Flow Rate	.67 gpm	Distance to Probe	25 cm.
Tank Mix	Foray 48B Undiluted	Sample Interval	1 sec.
RPM	10000	Number of Samples	60
FILE: C:\PMS\DATA\04169014.000		Number of Sample Rings	6

Number of Tests Combined: 3

UPPER LIMIT	N(RAW)	N/SEC	Gm/SEC	% N	% VOL.	ACCUMULATED % N	ACCUMULATED % VOL.
56	1688	4.81E+06	0.16	35.27	0.58	35.27	0.58
89	5495	2.06E+06	0.41	15.14	1.51	50.41	2.09
122	4583	1.90E+06	1.16	13.97	4.25	64.38	6.35
154	2683	1.30E+06	1.78	9.52	6.53	73.89	12.88
187	2139	1.26E+06	3.26	9.24	12.00	83.13	24.88
220	1651	1.00E+06	4.39	7.37	16.17	90.50	41.05
252	1048	553922	3.77	4.06	13.89	94.56	54.94
284	739	365232	3.67	2.68	13.50	97.24	68.45
318	388	185300	2.66	1.36	9.81	98.60	78.25
351	197	84830	1.66	0.62	6.11	99.23	84.36
382	108	39253	1.00	0.29	3.69	99.51	88.05
414	90	24537	0.81	0.18	2.97	99.69	91.02
447	63	16280	0.68	0.12	2.50	99.81	93.52
479	39	9945	0.52	0.07	1.90	99.89	95.41
512	32	7138	0.45	0.05	1.67	99.94	97.08
545	16	3813	0.29	0.03	1.08	99.97	98.17
578	12	2423	0.22	0.02	0.82	99.98	98.99
611	6	1218	0.13	0.01	0.49	99.99	99.48
644	2	477	0.06	0.00	0.23	100.00	99.71
677	1	344	0.05	0.00	0.19	100.00	99.90
710	1	161	0.03	0.00	0.10	100.00	100.00
TOTAL	2.10E+04	1.36E+07	27.17				

TOTAL ACCEPTED RAW PARTICLES / TOTAL IMAGES = 20980/ 32925.67 = 63.7%

NUMBER MEAN DIA.= D_{10}.... 109.64 µm
VOLUME MEAN DIA.= D_{30}.... 156.20 µm
SAUTER MEAN DIA.= D_{32}.... 212.92 µm

NUMBER MEDIAN DIA.= $D_{N.1}$... <56 µm
$D_{N.5}$... 88.37 µm
$D_{N.9}$... 217.66 µm

VOLUME MEDIAN DIA.= $D_{V.1}$... 140.05 µm
$D_{V.5}$... 240.76 µm
$D_{V.9}$... 403.68 µm

RELATIVE SPAN= 1.09

MEMBRANE FILTER HOLDERS

Purpose: To hold filter media.

General Description: Several holders have been designed to support membrane filters and other sheet fiber materials for sampling airborne particles. Most holders consist of a cylindrical body, one end of which is adapted for connection to a vacuum source. The other end is fitted with a circular supporting screen or carbon pad. A clamping device seals the filter between the supporting screen and an inlet cup to prevent bypassing of the sampled aerosol. When the inlet cup is fitted with an adapter, samples may be taken in a closed system or from an air duct. One type of holder (fig. 45) is designed to accommodate both the unringed and the ringed membrane filter (filter mounted on a plastic ring). The plastic ring holds the filter against the supporting screen and eliminates the need for an inlet cup. The materials of construction and sealing devices vary with designs.

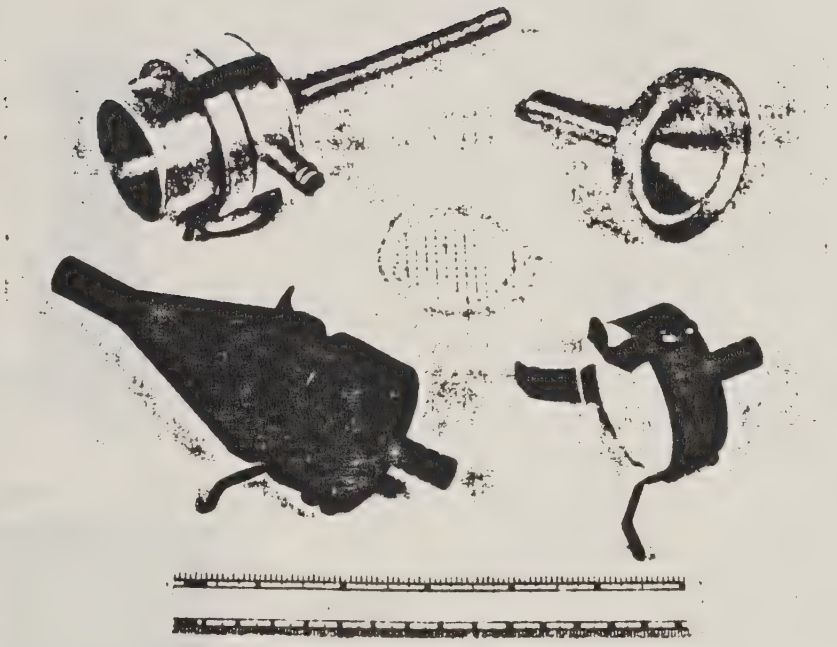

Figure 45.

Sources: Millipore Filter Corp., 86 Pleasant Street, Watertown 72, Mass. Gelman Instrument Co., Chelsea, Mich. Drawings of the membrane filter holder (fig. 45) available from Technical Development Laboratories, Communicable Disease Center, U.S. Public Health Service, P.O. Box 769, Savannah, Ga.

Reference: 76.

From: Wolf, H.W., P. Skaliy, L.B. Hall, M.M. Harris, H.M. Decker, L.M. Buchanan, and C.M. Dahlgren. 1959. Sampling Microbiological Aerosols. Washington: Public Health Service.

Figure 2. Membrane filter holders similar to the Wagner sampler.

Figure 3. Basic Rotorod sampler motor.

Figure 4. Field mounted Rotorod sampler - taped. The Rotorod sampler can be temporarily fastened with glass filament tape to any convenient stake or post.

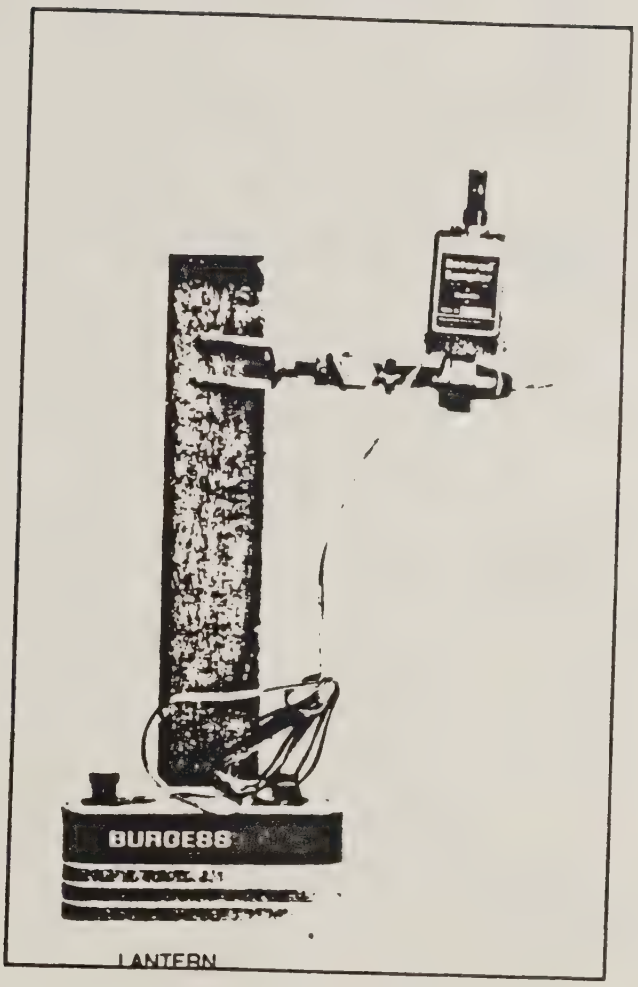

Figure 5. Rotorod sampler powered by 12-volt battery.

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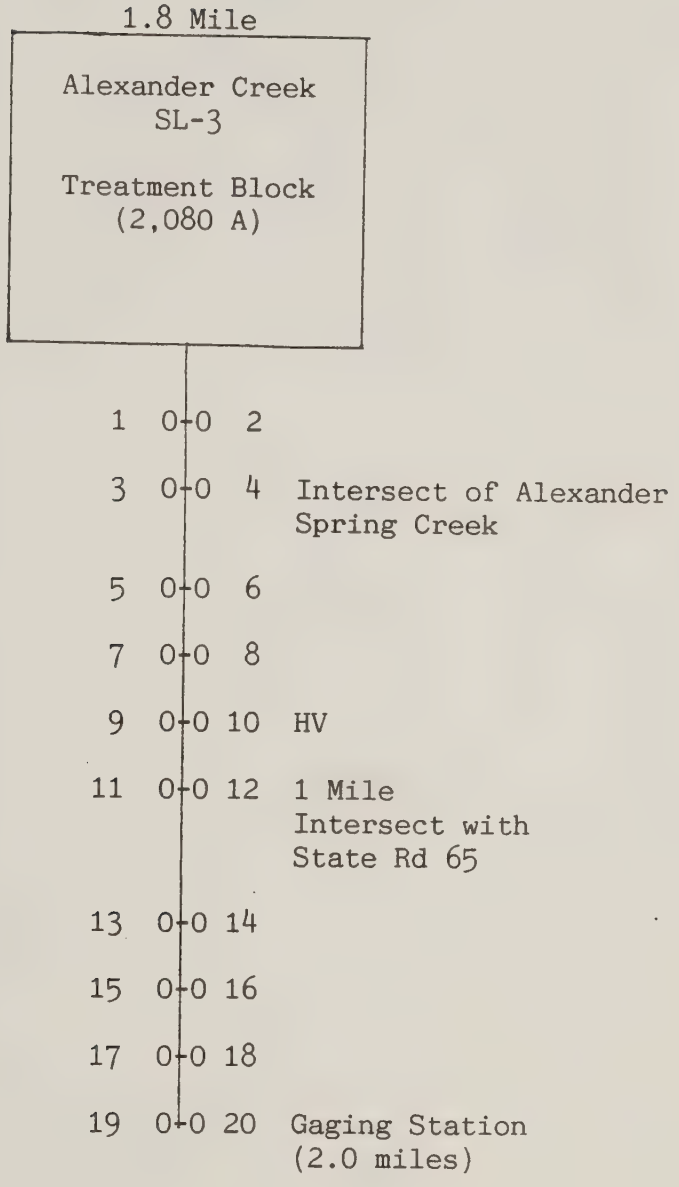


HV High Volume Sampler
0-0 Paired sampling station
Met tower

|_____|
Mile

Figure 6. Generalized diagram of downwind sampling and weather station array, not to scale.

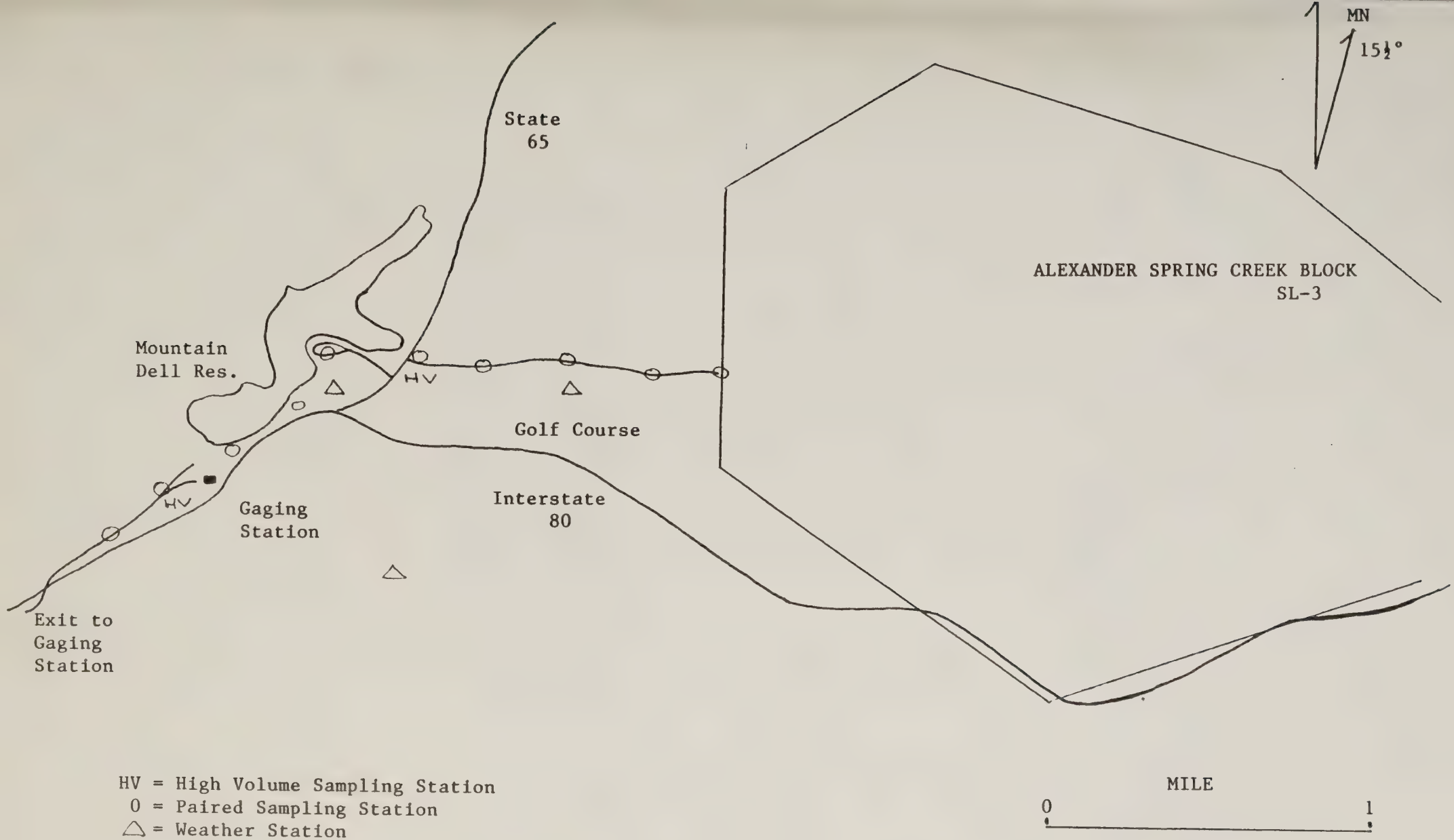
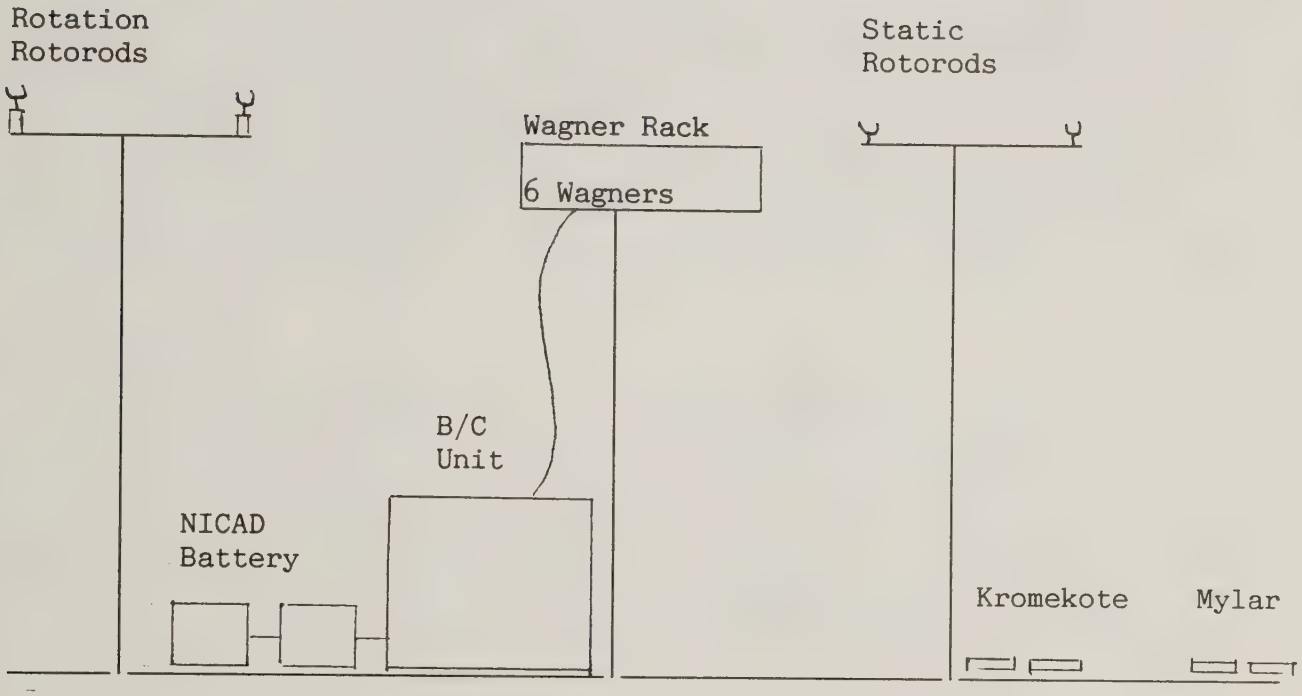


Figure 7. Location of downwind sampling stations and weather station, approximately to scale.



Notes: Samplers (Wagner, Rotorods, Kromekote and Mylar) at each station should be positioned perpendicular to axis of the spray cloud to avoid one sampler apparatus shielding the next.

Static Rotorods arms will be oriented perpendicular to drainage wind.

Wagners opening will point to ground.

Figure 8. Diagram of a sampling station.

The Test Officer log and report will record when each sampler is set out, picked up, activated, and deactivated.

Wagner Sampler

The Wagner sampler is similar to the holders shown in Figure 2. The Wagner supports a synthetic membrane filter, backed by a wire screen on the down flow side. Specific type of filter will be determined by the Laboratory Officer. Six Wagners will be used at each sampling station, the first and last Wagner should have negative counts indicating that the entire spray cloud passage was sampled. Combined the counts will provide a total dose. The Wagner will sample 12.5 liters of air per minute, supported by the DPG B/C sampling unit that contains vacuum pumps, and control switches (Figure 9). The Wagner opening will be pointed toward the ground. The unit will use 2 each 12 volt NICAD batteries. The B/C units will be operated manually or remotely at the direction of the Test Officer.

Rotorod^{R} Sampler

The "U"-shaped brass Rotorod sampler, developed by Metronics, and currently produced by Ted Brown Associates, is a rotating arm impaction device capable of obtaining quantitative data of airborne particulates in the size range $\geq$ 10 microns. At a nominal 2400 rpm which moves the collecting surfaces through the air and thus causes particles within the air intercepted by the collector rods to become impacted on the leading flat-surfaced edges of the rods. It samples 120 liters per minute when rotating at 2400 rpm. The collecting surface of the "U"-shaped rod is 0.159 cm. Its basic components are a constant speed motor and aerodynamically designed collector rods which are rotated by a 12-volt motor (Figure 3). The reference (Ted Brown Associates, 1976 and Edmonds, 1972, and Flottum, 1984) describe the sampler and provide instructions for its installation, operation and evaluation.

The "U"-shaped Rotorod samplers will be used at each sampling station. The Rotorod will be rotated to sample airborne B.t. particles through impaction. Rotorods will also be used statically to collect particles impaction caused by moving air. All Rotorods will be elevated 1.5 meters above ground.

The Rotorods will be used in duplicate at each station, 2 rotating and 2 static. The rotating Rotorods will be connected to a 12-volt motor and powered by a 12-volt Burgess or comparable battery. The Rotorods will be operated during the same time as the Wagner samplers.

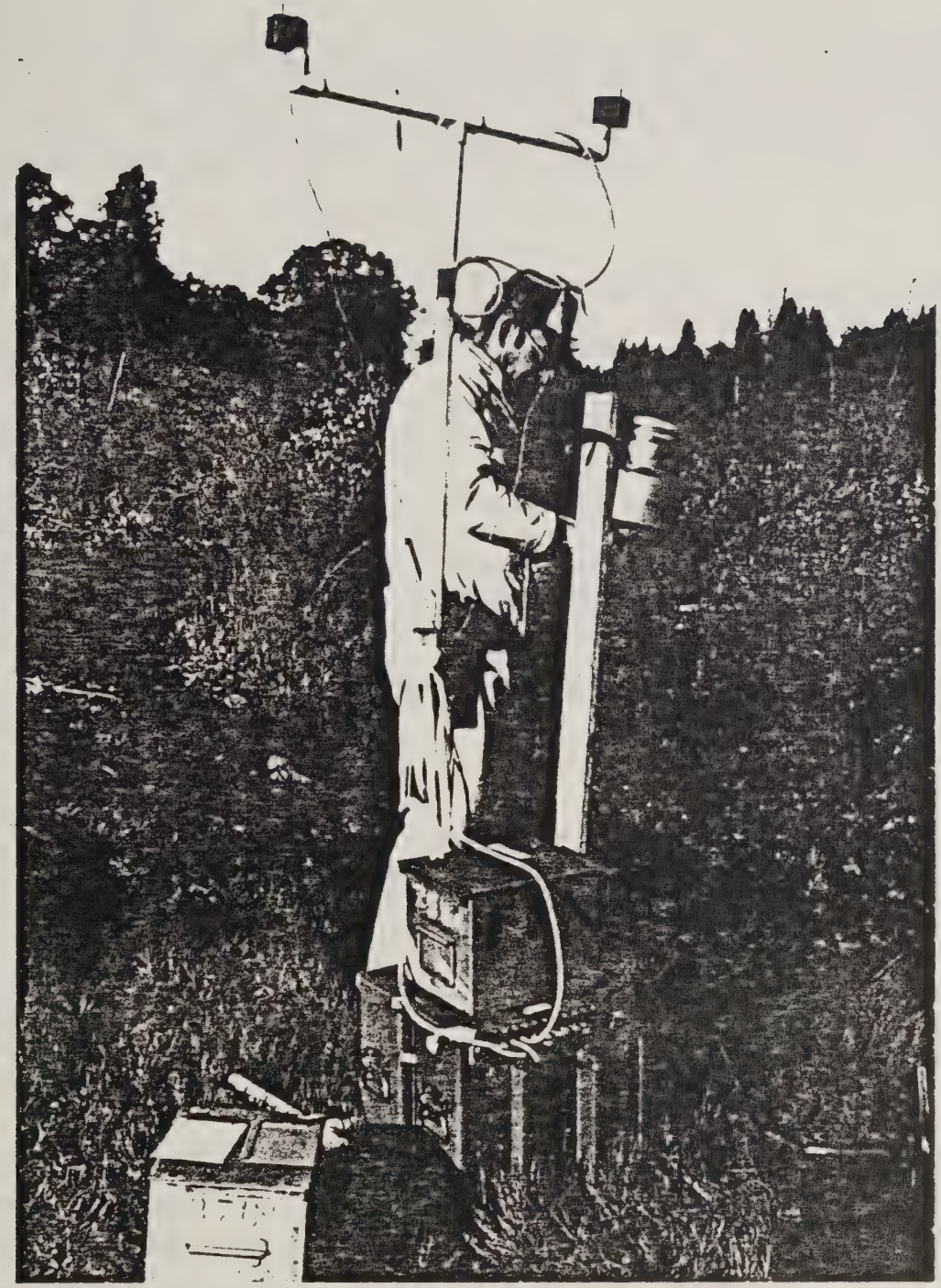


Figure 9. B/C sampling unit with NICAD battery, vacuum tubing, and Rotorod motors.

Kromekote Card

Kromekote cards, measuring 4 5/16 x 6 9/16 inches, will be placed at each sampling station to collect particles that might deposit by gravitational settling. The cards will be placed in plastic holders and positioned horizontally at ground level in duplicate, on a flat piece of plywood. Kromekote cards and holders will be provided by the USDA Forest Service.

Mylar Sheet

Mylar sheet, measuring 4 5/16 x 6 9/16 inches also will be positioned at ground level in duplicate near the Kromekote cards. The Mylar will also collect deposition resulting from gravitational settling. Mylar will be provided by the USDA Forest Service.

Controls

Field control samples will be used in addition to laboratory controls specified in laboratory standard operating procedures. Controls will be set up and operated (Wagners aspirated and Rotorods rotated) for 10 minutes at every even numbered sampling station. This will be done when the sampling station is set-up and before spray release begins. Controls will be packaged and removed from the sampling area prior to spraying. A Mylar sheet control sampler also will be placed at every even numbered sampling station and picked up prior to commencement of spraying.

Quality Control

Prior to the study the DPG Laboratory Officer will evaluate a fresh sample of FORAY 48B to become familiar with its physical and biological properties to the extent these factors might influence laboratory analyses. The Laboratory Officer will also evaluate suitability of two growth media recipes provided in the Appendix. Applicable DPG standard operating procedures will be followed including quality control and use of control samples. Data report should include results of laboratory control samples. Quality control includes both handling and exposure of control samplers and samples to detect B.t. background, natural and accidental contamination of samples by FORAY 48B.

A liter of tank mix that represents the batch of FORAY 48B applied to SL-3 will be collected from the spray aircraft by the Air Operational Officer and sent to the DPG laboratory for analyses. The sample should be placed in a plastic bottle and double packaged in plastic bags.

Avoiding Contamination

The most serious threat to the integrity of the sampling is contamination of samples with B.t. B.t. is a spore former that occurs naturally in the soil. Being a spore former it is persistent as opposed to vegetative cells that are susceptible to UV radiation and other degrading factors. After treatment the study area including foliage , soil, and other surfaces will be contaminated. Potential sources of sampler contamination include contaminated equipment, (sampling equipment, vehicles, containers) non-sterile samples, secondary

cards, measuring 3.10 x 8.0 inches,
statted to collect particles
The cards will be placed
ground level in
te cards and holder, will b

be ... weekly

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... samples, ... placed in ... lab... icary ... (Wh...
... before ...
the samples ...
... placed in ...
amount of

... to study the ... lead ... 1972 ...
... used to reduce ... ... particles and
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Appendix ... standard operating ...
quality ... of control ...
In ... procedure ...
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... the beads ...
... for ...
... samples will be placed in plastic bags

The integrity of the sampling ...
... a format that occurs ...
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aerosols (natural and man made), improper handling, packing, transportation of samplers, and contaminated crews (skin and clothing). Potential of contamination beginning of first trial contamination of equipment from DPG is minimal as B.t. has not been used on the DPG ranges nor in their laboratories. But once B.t. is released contamination is a serious threat. This applies to sampler pick up after the first trial. Suggested procedures to reduce potential for contamination include:

1. Sterilize samplers - Wagners and Rotorods.

2. Label samples in accordance with plan.

3. Wear clean clothing daily.

4. Avoid creating dust and secondary aerosols near samplers.

5. Approach samplers on downwind side.

6. Scrub Kromekote card holders to remove any B.t. contamination.

7. Handle Mylar with sterile instruments (e.g. forceps) or clean gloves remembering once the exposed Mylar is touched the instrument or glove is contaminated.

8. Follow laboratory officers instructions on handling and transporting samplers.

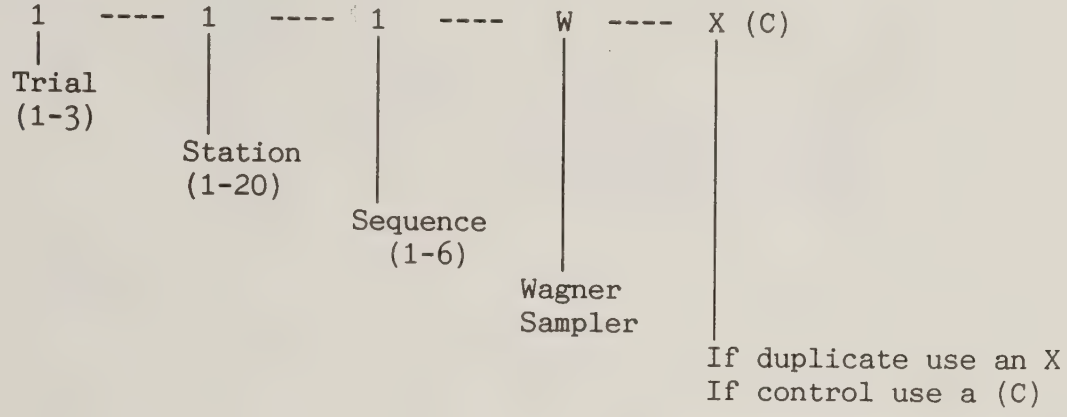
9. Keep vehicles and personnel upwind during trials - avoid the spray cloud.

10. Wash-down vehicles, B/C units, batteries, racks, and tote boxes. This will help to reduce potential of contamination from secondary aerosols and cross contamination.

Sampler Marking

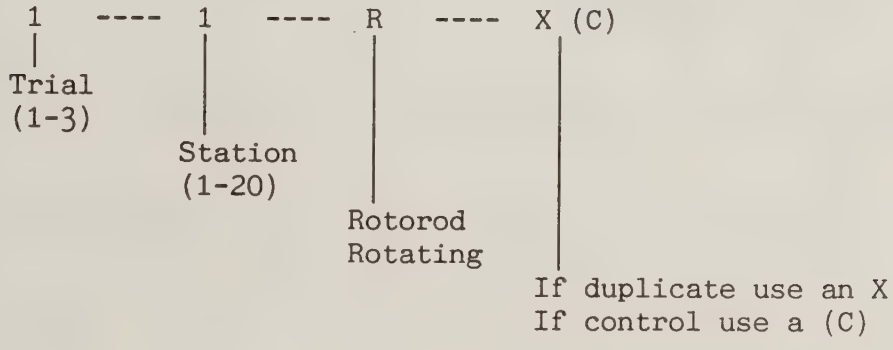
Sampler marking codes will be used throughout the study - that is from the laboratory where the sampler is prepared through to the reporting of data.

a. **Wagner**



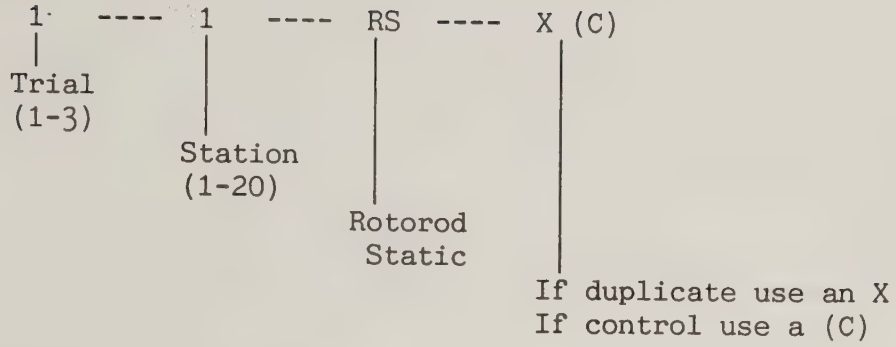
Wagners marking code will be placed on tape stuck to the Wagner.

b. **Rotorod (Rotating)**

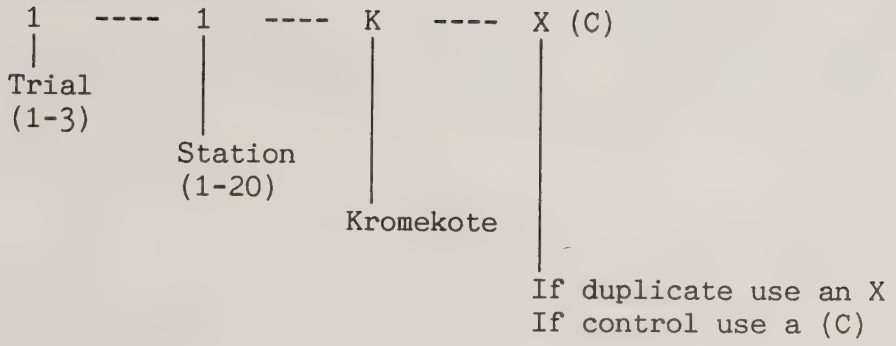


Marking codes will be placed on outside of the Ziplock bag and not directly on the Rotorod.

c. **Rotorod (Static)**

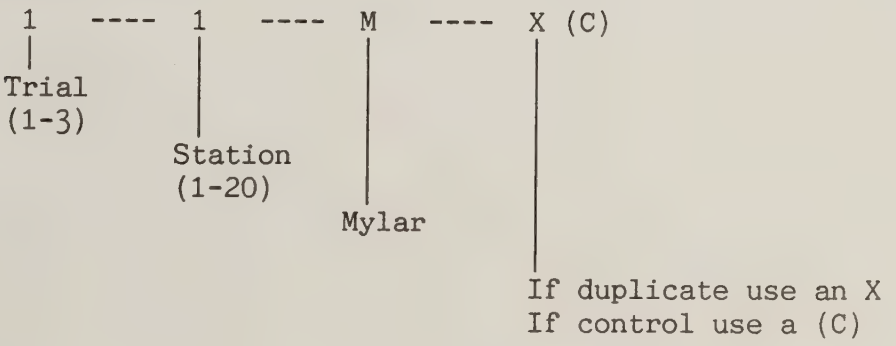


d. **Kromekote Card**



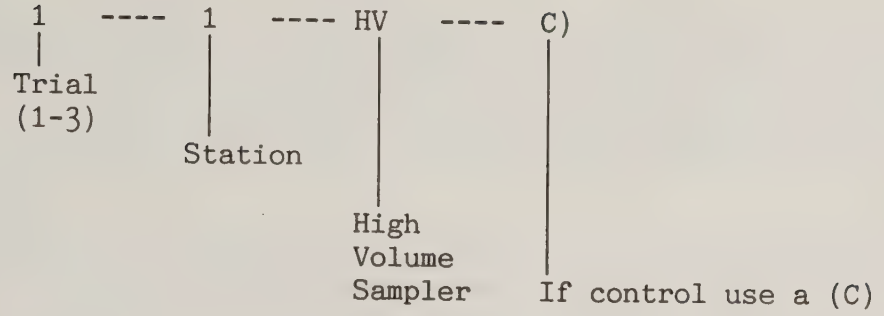
Kromekote cards will be marked on the bottom edge with a small marker.

e. **Mylar Sheet**



Marking codes will be placed on the outside of the container used to collect the Mylar and not directly on the Mylar.

f. **High Volume Air Sampler**



Sampling Equipment Requirements

There will be 10 duplicate sampling stations numbered 1-20 (Figure 6). The duplicates will be approximately at the same distance downwind but may be separated from 10 to 50 meters from each other. Samplers and samples from duplicate stations will be considered duplicates. DPG will provide all equipment and samplers except the Forest Service will provide:

Equipment (Per Trial)

	Required	Controls	Spare
B/C Sampling Units w/Wagner rack	20	--	3
NICAD battery	40	--	5
Generator, 110-V, AC	2	--	1
Rotorod motors w/bracket	40	--	10
Stands for Wagners and Rotorods	20	--	2
Kromekote card holders	40	--	10
Burgess 12-V Battery (unless powered by B/C)	20	--	20

Samplers (Per Trial)

Wagners	120	10	12
Rotorods	40	10	12
Kromekote cards	40	--	12
Mylar sheet	40	10	12
High Volume	2	2	0

Laboratory Assay

Wagners. Membrane filter will be dissolved in an appropriate fluid, and diluted and plated out on an appropriate growth media to be determined by the DPG Laboratory Officer, (note 2 separate growth media recipes are provided in Appendices). Data will be reported by colonies per sample. Excess collecting fluid will be retained for additional assay as required and possibly for assay at another laboratory.

Rotorods. Rotorods will be retrieved from the motor by covering the Rotorod with a Ziploc bag and sealing the zip without directly touching the rod. Labels will be placed on the outside of the Ziploc bag and not on the Rotorod. In the DPG laboratory the B.t. will be extracted from the Rotorod and the Ziploc bag, and the diluent will be diluted and plated. Excess collecting fluid will be retained for additional assay as required.

Kromekote Cards. Cards will be examined for presence of the honey colored FORAY 48B stains. If stains are visible the cards will be assayed for number and size of stains, and the resulting data will be processed through the ASCAS program to provide tabular output of volume and mass recoveries. The Forest Service, Davis, California will assay the cards.

Mylar Sheets. Mylar sheets will be retrieved in the field by sterile forceps and placed in sterile glass bottles or Ziploc bags. B.t, will be extracted and plated-out in the same manner as the Rotorods at the DPG laboratory. B.t. deposition could be high at those stations closest to the treatment block.

Laboratory data should be provided to Study Director by July 15, 1991.

Weather Instrumentation and Measurements

Three EMCOT solar powered weather stations (Ekblad et al., 1990) will be used to collect wind speed, wind direction, temperature, and relative humidity. Sensors will be positioned at 4 feet and 20 feet levels. Specifications of EMCOT include:

- Capable of being erected by one person
- Transportable in a sedan or pickup
- Real time screen display
- High frequency data, greater than 2/second
- Store 8 hours of data
- Display events graphically on the computer screen or printer
- Two temperature sensors
- Relative humidity sensor
- Fast response vertical windspeed
- Horizontal windspeed and direction

The weather stations will be deployed at strategic locations within the study area. Wind data will be collected at 2-second intervals to provide turbulence data by the FSCBG model. Data collection will begin 15 minutes prior to beginning of spraying and continue for two hours after spraying.

Specific location of the three stations will be decided upon consultations with project meteorologist.

The stations will be installed, operated, and maintained by an engineering technician from the USDA-FS Missoula Technology Development Center (MTDC).

MTDC will provide computation of all weather data in a data report by July 15, 1991.

Field Data Requirements

Data requirements and person responsible for providing the data are listed below. These data will be needed for each of the three treatments of Block SL-3.

1. Operational Treatment Data (Andy Knapp)

   Date
   Aircraft
   Pilot(s)
   Time block treatment began
   Time block treatment ended
   Total gallons applied
   Total acres treated
   Description of block treatment - specifically where the spray swaths were applied and when (Example Figure 10).
   Other remarks from pilot

2. Aerial photographs of Block SL-3 (Bill Klein)

3. Map of sampling stations and EMCOT weather stations showing measured location (Don Lassila)

4. Weather data from 3 EMCOT stations collected at 2 heights (2 meters 6.5 meters) (Don Lassila)

   Wind speed
   Wind direction
   Temperature
   Relative humidity
   Cloud cover
   Barometric pressure (from National Weather Service, Salt Lake City airport)

5. Sampling (DPG Test Officer)

   Time samplers set out
   Time samplers picked-up
   Time samplers were activated
   Time samplers were deactivated
   Time control samplers were activated, and deactivated, set out, and picked up

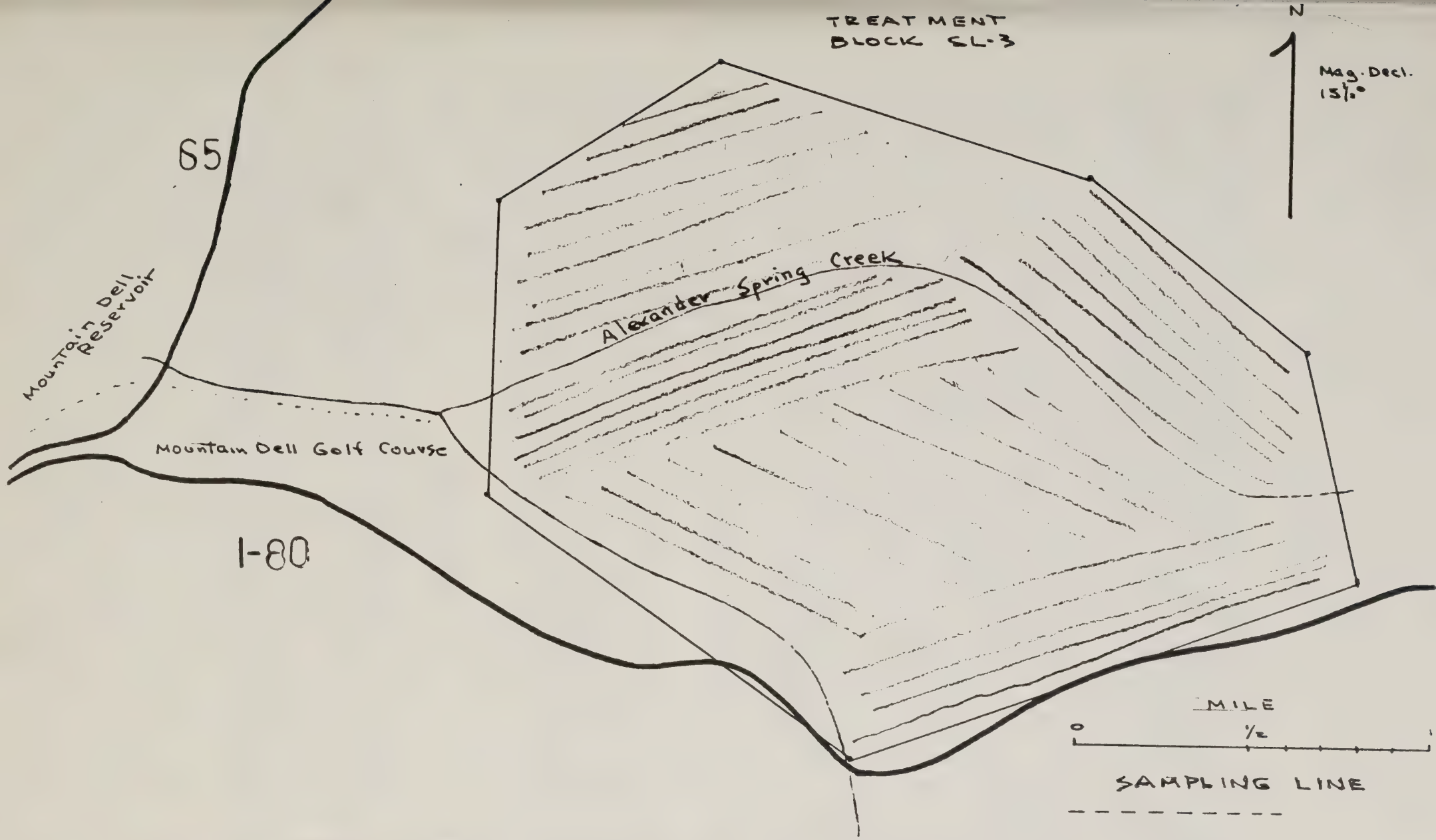


Figure 10. Example of diagram of how each swath was applied to treatment block SL-3.

4-24-91
JWB

[illegible]

DATA ANALYSES

Data analyses, initially, will approach each task separately, and as appropriate integrate results and analyses. Data from the laboratory and field controls will be analyzed and considered in the analyses and discussed in the report. Duplicate samplers have been included in the design to increase confidence as field sampling is recognized as being inherently highly variable. A statistician will be contracted to analyze these data and to assist in preparing the data analyses section of the report and manuscript.

Task 1. Laboratory results will be compared and analyzed to determine a level of confidence for the types of samplers used and the resulting samples. Analyses will address questions to include: are recoveries within expectations and model predictions for given observed weather conditions and downwind distances; are recoveries relatively consistent; are the Wagner recoveries more or less consistent than the Rotorod recoveries; and what are the differences (significant) among duplicates at same station and same downwind distances for the Wagner and Rotorods? The Kromekote card will be evaluated qualitatively only, as recoveries are dependent upon observing stains left by depositing spray drops. Positive cards are not expected at distances greater than 0.5 miles from the downwind edge of the spray block. Deposition on the Mylar sheets will be evaluated statistically by comparing duplicate recoveries as a function of downwind distance, and to FSCBG model predictions. The Mylar assay is more sensitive thus positive recoveries are expected at most stations.

Task 2. Analyses under Task 2 will overlap that of Task 1 but focus on quantitative data and provide statements of statistical confidence in the quantitative recoveries.

Task 3. FSCBG model runs will be made after the field studies are completed. Input to the model will be the conditions existing during spraying supplemented by estimates of non-measured conditions e.g. height of the mixing layer and winds at spray release height. A statistical analyses of predictions among trials will be made along with significant differences that might be noted when input parameters are changed or modified during the FSCBG sensitivity analyses.

COORDINATION

Treatment Supervisor - John Anhold (801) 625-5292, FTS 586-5292
Responsible for overall conduct of the eradication program and providing manpower as requested.

Public Affairs Officer (PAO) - L.J. Western (801) 524-6207, FTS 588-6207
Responsible for all public affairs activities to include press releases, media contacts, public inquiries related to the program and studies, and coordination with Dick Whitaker, DPG PAO.

Study Director - Jack Barry (916) 758-4600, FTS 460-1715
Responsible for planning, coordination, documenting and reporting of the off-site spray movement study.

DPG Scientist - Bruce Grim (801) 831-3371
Responsible for all coordination administration and support with U.S. Army Dugway Proving Ground to include coordination between PAO and Dick Whitaker, Dugway public affairs officer and U.S. Army Aberdeen Proving Ground; and coordination with Dugway's Lockheed contract.

DPG Project Officer - Gary Sutton (801) 831-5638
Responsible for coordination with Lockheed contractor for Test Officer and field crews.

DPG Laboratory Officer - Lloyd Larsen (801) 831-5173
Responsible for preparation of samples, laboratory assay of samplers, quality control procedures, and reporting data.

Test Officer - Todd Warr (801) 831-5335
Responsible for set-up operation, and pick-up of sampling equipment, and quality control, and reporting on field operations.

Utah State Liaison Officer - Mark Quilter (801) 538-7190
Responsible for liaison, coordination between State and local jurisdictions, and study personnel.

Continuum Dynamics, Inc. - Dr. Milton Teske (609) 734-9282
Responsible as a contract consultant for evaluation of FSCBG model and comparing model predictions to observed data.

NOVO Nordisk Company - Temple Bowen (203) 790-2632
Responsible for coordinating NOVO activities and support.

Project Engineer - Bob Ekblad (406) 329-3988
Responsible for providing meteorological support and EMCOT weather stations, and weather data report.

Air Operations Officer - Andy Knapp (208) 364-4222
Responsible for providing data requested in paragraph 1 of Field Data Requirements.

Mechanical Engineer - Don Lassila (406) 329-3988
Responsible for operating EMCOT weather stations, recording and reporting data, and preparing map of study area.

SAFETY

Safety is everyone's responsibility both in practice and in reporting real and potential hazards. All personnel involved in this study will be familiar with and observe procedures outlined in the Operational Project Safety Plan. Supervisors are responsible to insure that personnel read the Safety Plan and all personnel are responsible for safe work practices. The primary safety hazard is driving on Interstate 80, particularly access and egress; travel on unimproved roads; operating vehicles and equipment during early morning conditions; and lifting of equipment. EMCOT weather stations will be located to avoid electrical wires and vehicle and foot traffic. Guy wires and stakes will be marked with fluorescent engineering tape. Weather stations may be fenced if safety and security is deemed to be a problem. The material safety data sheet and pesticide label for FORAY 48B are in the Appendix.

REPORTING AND TECHNOLOGY TRANSFER

Results of this test and the analyses will be reported in a joint USDA Forest Service/U.S. Army report. If the field and laboratory procedures are successful, a manuscript will be prepared and submitted to the American Society of Agricultural Engineering or the Journal of Applied Meteorology for publication. In addition, a paper will be offered for presentation to the summer or winter meeting of the American Society of Agricultural Engineers. Applicable results will be incorporated in FS training sessions directed at persons who develop environmental impact studies, and who plan and conduct aerial and ground spray operations.

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1. Material Safety Data Sheet - FORAY 48B
2. FORAY 48B pesticide label
3. Recipe for media to culture FORAY 48B (From David Hobbs, NOVO)
4. Recipe for media to culture FORAY 48B (From Roy Beckwith)
5. Reprint - Collection Efficiency of Rotorod Samplers for Sampling Fungus Spores in the Atmosphere by Robert L. Edmonds
6. Reprint - A Quantitative Sampling Method for Airborne Sweet Corn Pollen Under Field Conditions by P.K. Flottum, et al.

APPENDIX

[illegible] Safety Data Sheet – [illegible]

[illegible]

For media to culture [illegible]

For media to culture [illegible]

[illegible] - Collecting [illegible] History of Rotenone Studies [illegible]
Species [illegible] by Robert [illegible]

Report [illegible] - A Quanti[illegible] [illegible]
Pollen [illegible]

Novo Laboratories, Inc.
33 Turner Road
Danbury, CT 06810-5101

Emergency Phone Number: (203) 790-2600
Chemtrec Number: (800) 424-9300

MATERIAL SAFETY DATA SHEET

DATE: May 5, 1988

REVIEWED: 5/88

I. IDENTIFICATION

PRODUCT NAME: Foray 48B™

CHEMICAL NAME: *Bacillus thuringiensis* Berliner var. *kurstaki*

FORMULA: NA

CHEMICAL FAMILY: Biological insecticide

MOLECULAR WEIGHT: NA

SYNONYMS: NA

DEPARTMENT OF TRANSPORTATION:

HAZARD CLASSIFICATION: None

SHIPPING NAME: None

FREIGHT CLASSIFICATION: Insecticides, Agricultural, Liquid N.O.I.

CAS NUMBER: 68038-71-1

CAS NAME: *Bacillus thuringiensis* Berliner var. *kurstaki*

II. PHYSICAL DATA

BOILING POINT, 760 mm Hg: NA

SPECIFIC GRAVITY (H_2O = 1): 1.16

VAPOR DENSITY (AIR = 1): NA

PERCENT VOLATILES BY VOLUME: NA

APPEARANCE AND ODOR: Flowable liquid concentrate, characteristic fermentation aroma

POUR POINT: NA

pH: 4.0 - 4.5

VAPOR PRESSURE AT 20°C: NA

SOLUBILITY IN WATER, % BY WT.: Suspendable

EVAPORATION RATE (BUTYL ACETATE = 1): NA

III. HAZARDOUS COMPONENTS

MATERIAL	%	TLV (Units)	HAZARD
Bacillus thuringiensis Kurstaki	15	None	None known

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (TEST METHODS): NA

FLAMMABLE LIMITS IN AIR, % BY VOLUME: NA

EXTINGUISHING MEDIA: No special requirements

SPECIAL FIRE FIGHTING PROCEDURES: No special requirements

UNUSUAL FIRE AND EXPLOSION HAZARDS: None known to exist

V. HEALTH HAZARD DATA

TLV AND SOURCE: None established by ACGIH or OSHA

ACUTE EFFECTS OF OVEREXPOSURE

SWALLOWING: None known

SKIN ABSORPTION: Not known to occur

INHALATION: None known to exist

SKIN CONTACT: None known to exist

EYE CONTACT: None known to exist

CHRONIC EFFECTS OF OVEREXPOSURE

Repeated exposure via inhalation can result in sensitization and allergic response in hypersensitive individuals.

CARCINOGENICITY:

NTP? No
IARC Monographs? No
OSHA Regulated? No

EMERGENCY AND FIRST AID PROCEDURES

SWALLOWING: Rinse mouth and throat with clear clean water.

SKIN: Wash with clear clean water.

INHALATION: Remove from exposure.

EYES: Flush with quantities of water.

VI. REACTIVITY DATA

UNSTABLE: STABLE: X CONDITIONS TO AVOID: None known

INCOMPATIBILITY (MATERIALS TO AVOID): None known

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: None known

HAZARDOUS POLYMERIZATION:

MAY OCCUR: WILL NOT OCCUR: X CONDITIONS TO AVOID: None known

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED: Dike and absorb spill with inert material (kitty litter, etc) and transfer to suitable container for disposal.

WASTE DISPOSAL METHOD: Waste disposal depends upon local requirements. Assure adherence to Federal, State and Local regulations subsequent to disposal.

VIII. SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION: None required under usual conditions of use. However, if exposure potential exists refer to NIOSH Criteria Guides to determine appropriate unit.

VENTILATION: Local exhaust as necessary to reduce, prevent, and control aerosol generation at source.

PROTECTIVE GLOVES: Rubber/neoprene EYE PROTECTION: Safety glasses or goggles

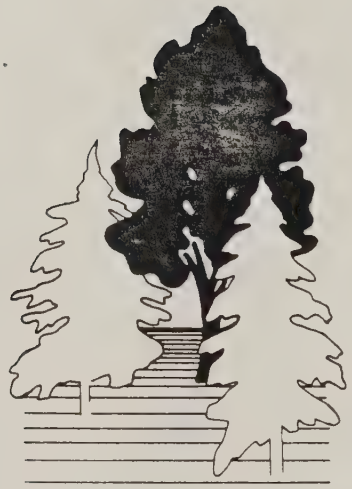
OTHER PROTECTIVE EQUIPMENT: As needed to prevent personal contact.

IX. SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING: Maintain good housekeeping, vacuum spills, avoid creating aerosol.

OTHER PRECAUTIONS: Long exposure of product to high heat and humidity may reduce product activity.

EPA REGISTRATION NUMBER: 58998-7



Foray™ 48B

Flowable Concentrate

KEEP OUT OF REACH OF CHILDREN

CAUTION

If in eyes, flush with plenty of water. Get medical attention if irritation persists.

ACTIVE INGREDIENT:		
	Bacillus thuringiensis Berliner var. *kurstaki*, primary powder fermentation product, potency of 12,600 IU per mg of product (equivalent to 48 Billion IU/gal.).	15%
	Inert Ingredient	85%
	Total	100%

DIRECTIONS FOR USE:

It is a violation of federal law to use this product in a manner inconsistent with its labelling.

FORAY 48B contains the spores and endotoxin crystals of *Bacillus thuringiensis kurstaki*. FORAY is a stomach poison and has high specific activity against lepidopterous larvae. After ingestion, larvae stop feeding within hours and die 2-5 days later. Maximum activity is exhibited against early instar larvae. FORAY 48B Flowable Concentrate may be used for both ground and aerial application. The product should be shaken or stirred before use. Add some water to the mix tank, pour the recommended amount of FORAY 48B into the tank and then add the remaining amount of water to obtain the proper mix ratio. Agitate as necessary to maintain the suspension. The diluted mix should be used within 72 hours

Ground Application: Use an adequate amount of tank mix to obtain thorough coverage without excessive run off. Use the recommended per acre dosages of FORAY 48B in the following amounts of water:

High volume hydraulic sprayers	100 gallons
Mist blowers	10 gallons

Aerial Application: FORAY 48B may be applied aerially, either alone or diluted with water, at the dosages shown in the application rates table. Spray volumes of 32-128 ounces per acre are recommended. Best results are expected when FORAY 48B is applied to dry foliage.

RE-ENTRY: FORAY 48B may be applied up to and including the day of harvest and in storage.

STORAGE AND DISPOSAL: Do not contaminate water, food or feed by storage or disposal of waste.

Storage: Store in a cool, dry place. Keep containers tightly closed when not in use. Store in temperatures above freezing and below 30°C (90°F).

Pesticide Disposal: Pesticide waste resulting from the use of this product may be disposed of on site or at an approved waste disposal facility in accordance with federal and local regulations.

Container Disposal: Triple rinse for equivalent, then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by incineration or, if allowed by state and local authorities, by burning.
If burned, stay out of smoke.

PRECAUTIONARY STATEMENTS: Hazardous to humans. May cause eye irritation. Avoid contact with skin, eyes, open wounds, or clothing. Wash thoroughly with soap and water after handling.

Environmental Hazards: Do not contaminate water by cleaning equipment or disposal of waste.

WARRANTY NOTICE: All goods supplied by Novo BioKontrol are of high grade and we believe them suitable for the purpose recommended but, as we cannot exercise control over their storage or use, no responsibility will be accepted by us for any damage or injury whatsoever arising from their storage, handling, application, or use.

APPLICATION RATES.

Crop	Pests	Rate* (pts/acre)	Dosage* (BIU/Acre)
Forests, Shade Trees Ornamenals, Shrubs, Sugar Maple Trees	Gypsy moth, spruce budworm browntail moth	1-1/3 - 3-1/3	8 - 20
	Tussock moths, pine butterfly, bagworm, leafrollers, tortrix, mimosa webworm, tent caterpillar, jack pine budworm, black headed budworm, elm spanworm, saddled prominent, saddleback caterpillar	1 - 2	6 - 12
	Red humped caterpillar, spring and fall cankerworm, california oakworm, fall webworm	0.5 - 1.0	3 - 6

*Use the higher recommended dose rate on advanced larval stages or under high density larval populations.

EPA Registration No: 58998-7
EPA Est No: 58998-DN-001

DISTRIBUTED BY:
Novo BioKontrol
A DIVISION OF NOVO LABORATORIES, INC.
33 Turner Road
Danbury, CT 06810-5101
(203) 790-2600

NET CONTENT **2.5**
(U.S. GALLONS)

Novo Nordisk

Entotech, Inc.

MEMO

TO: Jack Barry

CC:

FROM: DaH

DATE: April 18, 1991

RE: Forest Service - Drift Trials

Jack Barry
USDA Forest Service
Forest Pest Management
2121 C Second Street
Davis CA 95616

(916)-758-4600
-8181 (FAX)

Per your request the medium formula is as follows:

for one liter of formula:

Difco™ agar	15 grams
Difco ™ Nutrient Broth	8
water	one liter

No pH adjustment is needed.
Autoclave for 20 minutes at 121°C and 15 lbs. pressure.
Pour immediately into petri dishes.

After the plates have been exposed, growth can occur at room temperature or for faster growth, incubated at 30°C. Plates should be read in 12 - 48 hours.
Note: run a check plate to help identify Bt colonies, against contaminants.

The one liter sample can be picked up at your convenience, just call ahead to make sure I'm around.

David Hobbs

MESSAGE DISPLAY

To Barry, Jack:SCS06

From: Roy C. Beckwith:S26L05A ← telephone (503) 750-7363
Postmark: Apr 18,91 3:12 PM Delivered: Apr 18,91 3:18 PM
Status: Certified
Subject: Reply to: Culturing B.t.

Reply text:
From: Roy C. Beckwith:S26L05A
Date: Apr 18,91 3:12 PM
Jack--Here is the technique we use for needles--it should work for the wash from your impingement device. We use Tryptic Soy Agar for the media--the directions are on the box. Since the agar is autoclaved before use and we leave it for a short time-- we don't use any additives. I have never used the technique on Foray 48B; however, it is based on the HD-1 strain so should be the same as others we have used.
The technique is as follows:

1. Place 20 needles into a sterile test tube.
2. Add 20 ml of sterile distilled water.
3. Mix for 60 seconds.
4. Pour water into sterile test tube.
5. Make 1: 100 dilution (1 ml: 99 ml)
6. Mix for 60 seconds
7. Add 1 ml of dilute to sterile petri dish
8. Add about 10 ml of nutrient agar and mix contents by rotary motion (Agar is cool enough to be handled)
9. After agar gels, invert petri dish.
10. Leave petri dishes at room temperature from 24-48 hours.
11. Make spore counts (based on colonies) using a colony counter.

Preceding message:
From: Barry, Jack:SCS06
Date: Apr 18,91 1:39 PM
Roy, we are conducting a drift study in cooperation with R-4's gypsy moth project. Foray 48B will be used and we plan to use impingers to collect aerosols of the sample. The sampler effluent will be plated out to get a total count per unit of air sampled. What I need is your recommendation of growth media recipes that would be best suited for Foray and any additives that we might need to add to inhibit growth of contaminants. And any other thoughts. The Dugway Proving Ground lab will be doing this for us and they have a lot of

----------X----------

COLLECTION EFFICIENCY OF ROTOROD SAMPLERS FOR SAMPLING FUNGUS SPORES IN THE ATMOSPHERE

Robert L. Edmonds [1]

Abstract

In the sampling of fungus spores in the atmosphere, the collection efficiency, and thus the accuracy of the samplers for obtaining quantitative data, has rarely been considered for the particular fungus spore being sampled. This paper is designed to make potential users of Rotorod impaction aerosol samplers aware of the importance of considering sampling efficiency. A method for calculating efficiency is given.

Adequate study of the dispersion of fungus spores requires accurate sampling of the atmosphere. In many investigations the collection efficiency, and thus the accuracy of samplers, has not been considered for the particular type of fungus spore being sampled.

Suction devices and rotating arm impactors are the most common types of instruments used in the collection of fungus spores. Because of the large size range of fungus spores (from a few microns (μ) to 100μ), however, there is no one instrument yet developed that is capable of sampling the whole range with equal efficiency.

This paper proposes to indicate to users of rotating arm impaction samplers the importance of considering collection efficiency in sampling; to discuss theoretical aspects in determination of efficiencies; to provide an equation for determination of sampling efficiencies for various sizes of fungus spores; to demonstrate how collecting surfaces can be modified to in-

[1]The author is presently Program Coordinator, United States International Biological Program, Aerobiology Program, Botany Department, University of Michigan, Ann Arbor, Michigan 48104.

crease sampling efficiency; and to discuss the importance of the selection of a suitable sticky material for the leading edge of the sampler. The "Rotorod sampler" is used as an example of this type of device. It is commercially available and widely used.

The "Rotorod sampler" in the form developed and marketed by Metronics Associates, Inc. of Palo Alto, California (10) has been used for collecting fungus spores in the atmosphere by many workers including Asai (1), Froyd (5), Barksdale (2), Skilling (9), and Edmonds (4). It employs the process of inertial impaction with spores, and so forth, being impacted on a whirling arm.

Advantages of this sampler are low cost, simplicity, light weight, large sampling volume, suitability for experiments employing large numbers of simultaneous samplers, battery operation for remote locations, and the collection efficiency is not affected by wind speed up to 6.2 kph. The chief disadvantage is that collection efficiency is sharply dependent on spore size and density, and it can only be used for short periods of time because of over-loading of the collection surface.

Two sizes of Rotorods are available commercially from Metronics (Fig. 1). The U-shaped brass Rotorod has collection surfaces 1.59 mm in thickness, with arms 6 cm high, 8 cm apart and samples 120 liters per minute (lpm). It was designed to sample particles in the 15-25μ diameter range. The H-shaped chromel Rotorod is 0.38 mm in thickness, with arms 6 cm high, 12 cm apart and samples 41.3 liters per minute. It was designed to sample fluorescent particles (specific gravity 4.0 g cm^{-3}) in the 1-5μ diameter range (10). The Rotorod motors revolve at approximately 2400 rpm, with the collecting surfaces of the U- and H-shaped Rotorods revolving at 15.1 and 10.1 m sec^{-1}, respectively. The actual rpm for each motor varies and is supplied for each motor.

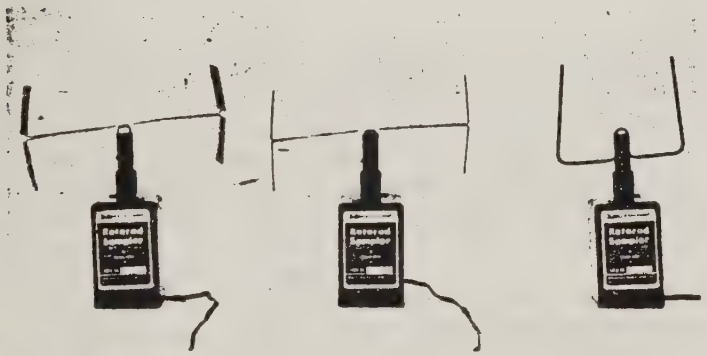


FIGURE 1. Rotorod samplers and motors. Commercially available Rotorods are the U-shaped (right) and H-shaped (center). Modified Rotorod is on the left.

DISCUSSION

Most researchers who are interested in collecting airborne spores usually wish to know the spore concentration in the atmosphere. Erroneous spore concentrations, however, can be calculated if collection efficiencies are not considered for the particular spore size under consideration. Not all spore sizes, even within the suggested range of the instrument, are collected with equal efficiency. Sampling sensitivity is low if efficiency is low, and low efficiencies also result in uneven distributions of spores on the collection surfaces (8). This is important to consider if sample fields are to be counted on the Rotorod arms.

Noll (8) has discussed theoretical and experimental aspects of whirling arm samplers. The collection efficiency (E) is largely a function of the particle parameter (P) (Fig. 2). Experimental data in this figure were obtained from a 16-stage rectangular collector impaction sampler developed by Noll. Each stage was designed to sample a specific size range of particles at 85-100% efficiency. The particular data used were derived from two stages designed to collect particles down to 26 and 13μ respectively. These stages had collection surfaces 3.2 and 0.8 mm in width, respectively, and revolved at 7.2 m sec^{-1}. Noll's data generally agree with experimental data of Chamberlain and Gregory for impaction of Lycopodium spores on cylinders (3). The efficiency suggested by Chamberlain for P equal to 10, however, is lower than those values suggested by Noll. Noll's data are preferred in practice because they are derived from a whirling arm sampler similar to the Rotorod, with similar sized rectangular collection surfaces, revolving at similar velocities. The line through the data was drawn as the line of best fit by eye.

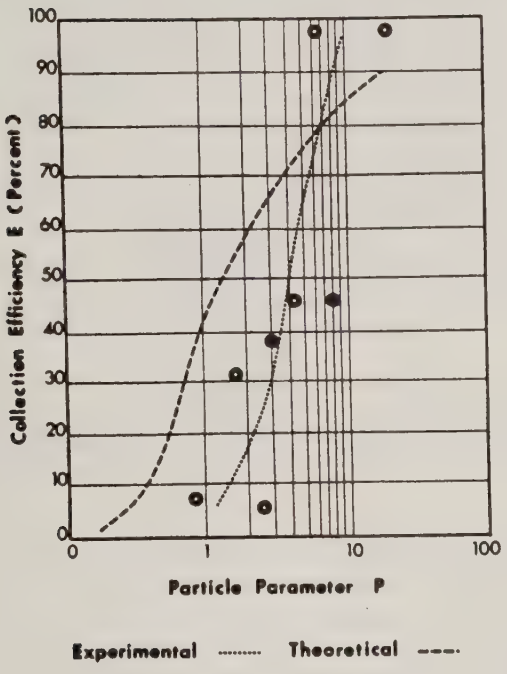


FIGURE 2. Relationship between particle parameter (P) and spore collection efficiency (E). (After (8)).

The theoretical curve presented in Figure 2 was determined by Langmuir and Blodgett for flow around a ribbon (8). Experimental values of E are lower than theoretically derived values, for P less than 7, but higher for P greater than 7. Chamberlain noted that experimental values of E were always lower than theoretical. He is uncertain whether this represents a fault in theory or a failure by sticky cylinders to retain all spores striking them. It would appear that the desired efficiency of 100% is approached as P approaches values of 10 or greater. In practice, because of the inconsistent agreement with theory, it is preferable to use the experimentally derived curve to determine E.

The selection of a suitable sticky material for the leading edge is important. If the surface is dry, particles bounce off. The material must be sticky, but if it is too thin, friction causes it to run off. If it is too thick, the edge loses its sharpness, the effective size of the collection surface is increased, and the collection efficiency is lowered. A 1:3 rubber cement and xylene solution used by Harrington, et al. (7), Froyd (5), and Edmonds (4) appears to give reasonable results.

The following is a general formula to calculate P for any spore size:

$$P = \frac{V_o \, d^2 \delta_p}{18 \, \eta \, L \, S}$$

P = Particle Parameter (dimensionless)
V_o = Average* Rotorod arm velocity (cm sec^{-1} U-shaped (1010); H-shaped (1510)
d = Diameter of sphere of equivalent volume to that calculated for the spore (cm)
δ_p = Density of spore (g cm^{-3})
η = Viscosity of air (poises, g sec^{-1} cm^{-1}), at 18°C = 182.7 x 10^{-6} poises
L = Width of rectangular collector (cm)
S = Dynamic shape factor of particle (dimensionless)

*Actual arm velocity is variable because rpm vary from motor to motor.
Actual rpm for each motor is supplied by manufacturer.
The value of E is read from Figure 2.

Fuchs (6) has suggested that S is 1.28 for ellipsoids with ratio of axes, major/minor = 4. For practical purposes, no great error is made by setting S = 1 for spores with ratio of axes less than 4.

Table 1. Values of particle parameter (P) and collection efficiency (E) of U-shaped Rotorods in spore sampling studies.

Author	Organism	Type of spore[a]	Average dimensions of spore (μ)	Diameter of spherical spore of equivalent volume (μ)	P particle parameter (from formula)	E Percent (from Figure 2)
Asai (1)	Puccinia graminis	Uredospore	24 x 18.5	22	9.4	95
Froyd (5)	Hypoxylon pruinatum	Ascospore	26 x 10.5	19	7.0	80
Barksdale (2)	Piricularia oryzae	Conidia	23 x 8.5	16	4.9	65
Skilling (9)	Scleroderris lagerbergii	Ascospore	19.5 x 5	12	2.8	30

[a]Spore density was assumed to be 1.0 and S = 1 (ratio of axes of spores is less than 4). Diameter of equivalent sphere is rounded to nearest whole number.

Asai, Froyd, Barksdale, and Skilling used U-shaped Rotorods in their experiments. Asai, however, was the only investigator to mention sampling efficiency, noting that spores in the vicinity of 20μ diameter are impacted at approximately 100% efficiency.

Table 1 shows values of P and E for each of the four studies. The Puccinia graminis spores trapped by Asai are impacted at close to 100% efficiency. The other spores are sampled at much lower efficiencies. Unit densities for spores was assumed.

The H-shaped Rotorods will impact spores of unit density down to 9μ in diameter at close to 100% efficiency. Thus, in selection of Rotorod size to be used, it is important that sampling efficiencies for both sizes of Rotorods be determined for the particular spore in question, in order to obtain maximum efficiency. If P is greater than or equal to 10, the efficiencies close to 100% can be obtained.

H-shaped Rotorods can also be modified (Fig. 1) to a particular efficiency by welding aluminum shims of different widths to the arms, thereby adjusting the width of the collection surface and increasing the collection efficiency. Welding of shims to arms increases the aerodynamic drag on the rotating arms, which probably results in small decreases in the rotation speed of the sampler perhaps of 5% or so. This was not checked in practice, but it should be considered. This modification shown in Figure 1 was used by the author to collect spores of Fomes annosus (4.5-5.0μ diameter)(4) at an efficiency similar to that of fluorescent particles (specific gravity 4.0 g cm^{-3}, 3.0μ average diameter) collected on H-shaped Rotorods. This enabled a direct comparison of their respective dispersion patterns, with the object of determining if fluorescent particles could be used for tracing spore dispersal.

Another factor to be considered in collection efficiency is that of possible changes in shapes and, thus effective sizes of fungus spores while they are airborne due to changes in moisture content, and so forth. This is difficult to assess and thus has not been considered in the calculations.

CONCLUSION

In sampling fungus spores with a rotating arm impaction device such as the Rotorod, it is important to consider the collection efficiency for the particular species of fungus spore being sampled in order to make accurate calculations of the concentration in the atmosphere. If the particle parameter P is calculated to be greater than 10, then the efficiency of collection is close to 100%. If P is less than 10, then the collection efficiency can be read from Figure 2.

An alternative to this is to modify the size of the collecting surface to increase collection efficiency.

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A QUANTITATIVE SAMPLING METHOD FOR AIRBORNE SWEET CORN POLLEN UNDER FIELD CONDITIONS[1]

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Abstract

The rate of pollen dehiscence in a sweet corn (*Zea mays* L.) plot was measured using a Rotorod Sampler®. Samples were taken from 0700 to 1230 h for 3 days during anthesis. Totals were averaged over the 3 days, and the resulting composite data were used to develop a sampling protocol accurate for determining the rate of pollen release. Results showed that a 10-min sampling period, with a frequency of at least once every half hour was required to accurately reflect the pollen release rate.

Additional index words: **Pollen, Dehiscence, Pollen emission profile, *Zea mays* L.**

STUDIES of pollen dehiscence in sweet corn (*Zea mays* L.), and many other grasses (Gramineae) have been primarily concerned with developing techniques that predict the date flowering will begin (Cross and Zuber, 1972; Gardner et al., 1981; Hanway 1966). In sweet corn the process of pollen dehiscence, usually defined only as anther decention, is fairly well understood (Knox, 1979; Percival, 1969). However, studies of patterns of pollen release for a single day or for the period of anthesis have generally been qualitative in nature, as quantitative in vivo measurements of airborne sweet corn pollen have not been made.

This paper 1) describes a method used to measure the pollen emission patterns in a flowering sweet corn field, 2) documents the effectiveness of the method, and 3) presents data pertinent to the optimal use of the method.

Materials and Methods

A Rotorod Sampler®[3], a rotating impaction device powered by a 12 v battery, was used to collect airborne sweet corn pollen. Airborne pollen is captured on the leading surface of removeable 64-mm plastic rods held by the rotating arms (Fig. 1). General Electric G-697 Silicone Grease®[3] facilitates capture and retention of the pollen grains. The arms are rotated at ca 2400 RPM, sampling a volume of ca. 120 L/min. Operating efficiency, or ability to collect airborne pollen in the volume sampled, was determined to be greater than 99% by manufacturers specifications.

These studies were conducted at Madison, Wis. during 1981. The Rotorod Sampler was positioned centrally in a 40 m^2 plot of 'Commander' sweet corn with the maximum height of the sampler rods slightly below center of representative tassels in the plot. Sample rods were replaced at the end of each sampling interval whereupon the number of captured pollen grains were counted.

Two concurrent collection procedures were designed and

Fig. 1. The Rotorod Sampler®, showing removable 64 mm plastic rods.

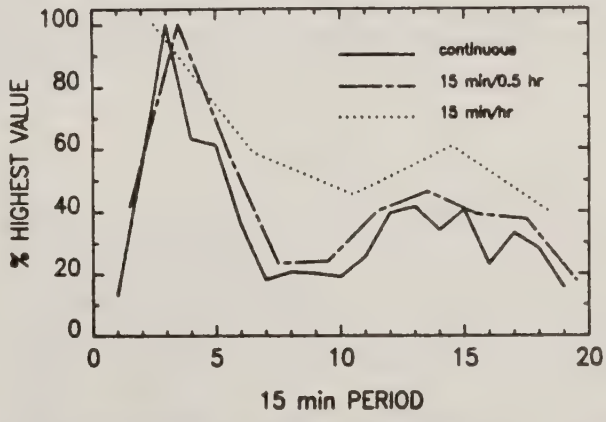

Fig. 2. Relationship of continuous, 1 sample/h and 1 sample/0.5 h profiles.

their results compared. The first procedure, subsequently referred to as the continuous method, consisted of running one Rotorod Sampler for 22 consecutive 15 min periods, from 0700 to 1230 h, for 3 days in the flowering sweet corn plot. Actual numbers of collected pollen grains were converted to pollen grains/L and plotted by period to develop a pollen emission profile for each day. The three daily profiles were then aligned so that the 15-min periods containing the initial daily peak coincided. This was done in order to accommodate the relative times of pollen dehiscence, not absolute time of day. Initial daily samples containing no pollen were not included in the analysis. Thus, the resulting composite profile consisted of 19, 15-min periods (Fig. 2).

To determine if reliable data could be obtained from fewer sampling periods than the 19 used to develop the composite of the continuous sampling method, the same data were reanalyzed assuming 1) one 15-min sample/h and; 2) one 15-min sample/0.5 h. For the one sample/h method, three groups of 15-min periods were analyzed as

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[3] Ted Brown Assoc., 26338 Esperanza Dr., Los Altos Hills, CA 94022. Mention of a trade name does not constitute a guarantee or warranty of the product by the USDA nor an endorsement over other products not mentioned.

if each group represented a distinct replication of the method. From Fig. 2, nonrandomized groups used were:

Group 1: periods 1,5,9,13, and 17
Group 2: periods 2,6,10,14, and 18
Group 3: periods 3,7,11,15, and 19

For the one sample/0.5 h, two nonrandomized groups were analyzed as distinct replications:

Group 4: periods 1,3,5,7,9,11,13,15,17, and 19
Group 5: periods 2,4,6,8,10,12,14,16,18, and 19[4]

Regressions of pollen emission on period were conducted for each of the three data arrangements (continuous, one 15 min sample/h and one 15 min sample/0.5 h) using orthogonal polynomials to represent period numbers.

The second collection procedure used consisted of running another Rotorod Sampler® for a period of only 10 min/sample. Samples were collected for the first 10 min of periods 1,3,5,7,9,11,13,15,17, and 19, (one 10 min sample/0.5 h), with the sampler left idle for the remaining 5 min of the period. These samples were neither randomized nor replicated. Results of these 10-min samples were analyzed as above and compared to the composite results and to the results of the one 15-min sample/0.5 h.

Results and Discussion

Method 1

Daily pollen emission profiles were strikingly similar. A large peak was recorded during one of the first 3 15-min periods each day. This was followed by a reduction in emission intensity, another slight increase then decreasing thereafter.

Regression analyses to determine the relationship between the amount of pollen collected and the time of day demonstrated significant linear ($P < 0.05$), quartic ($P < 0.01$), and quintic ($P < 0.05$) coefficients for each of the three daily profiles and the composite.

Regression coefficients for the one sample/0.5 h method, and for the composite profile (continuous method) were not significantly different (linear = -0.01 vs. -0.01; quartic = -0.004 vs. -0.002; quintic = 0.005 vs. 0.001, respectively - regression coefficients not converted from orthogonal polynomials). Similar analyses for the one sample/h method showed linear significance ($P < 0.05$), but neither the quartic nor the quintic coefficients were significant. Therefore, by inspection and analyses interpretation, it is shown that the curves for the continuous and one sample/0.5 h data arrangements are clearly high degree polynomials, while the curve for the one sample/h data arrangement is only a first degree relationship. These relationships are represented graphically in Fig. 2. Had sampling begun earlier each day, the initial peak may have been evident in the one sample/h arrangement, but this would not alter the lack of significance for the second peak.

[4] Nineteen used again for balance.

Method 2

Results of the second technique, running the sampler for a period of 10 min/0.5 h, were compared to the results of the continuous method and to those of the one 15 min sample/0.5 h data arrangement. These were not significantly different at the levels previously noted as there were no differences in the rate of pollen collection or shapes of the profile curves. Hence, one 10 min sample/0.5 h retained the accuracy of continuous sampling and displayed the bimodal emission profile of the sweet corn population. Further, this sampling frequency optimized accuracy of the data and reduced the effort required in data acquisition. Moreover, it permits one operator to gather data simultaneously from several locations in a large field.

Pollen release is dependent on several environmental variables (Flottum et al., 1983), as is the amount of pollen collected on the sampler. For this reason, use of the sampler cannot accurately determine when anther decention and subsequent pore formation occurs, but rather when the released pollen becomes airborne. In spite of this, the Rotorod Sampler®, when used in the manner described, is an accurate method of recording the pollen emission patterns in a sweet corn field.

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